Effects of Yeast and Bran on Phytate Degradation and Minerals in Rice Bread

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ABSTRACT: Rice bread is a potential alternative to wheat bread for gluten-sensitive individuals. Incorporation of rice bran into bread made from white rice flour adds flavor but also phytic acid, which can reduce the bioavailability of minerals. Breads with varied amounts of defatted bran and yeast were prepared to determine their effects on the phytate and mineral contents of the bread. A completely randomized factorial design was used with bran levels of 3.7%, 7.3%, and 10.5% of the dry ingredients and yeast levels of 1.6%, 3.2%, and 4.7%. Increasing the amount of bran decreased the phytate degradation from 42% at the lowest level of bran to 10% at the highest, and the amount of yeast had no significant effect. The bran contributed substantial amounts of magnesium, iron, and zinc. Breads with the lowest level of bran had phytate-to-zinc molar ratios between 5 and 10, which suggest medium zinc bioavailability. Rice bread is a tasty and nutritious food that is a good dietary source of minerals for people who cannot tolerate wheat bread.

Keywords: bran, bread, minerals, phytate, rice

Introduction

Bread is often referred to as the staff of life because it is a staple food that provides significant amounts of vitamins, minerals, and other nutrients to human diets. Most breads are formulated from a wheat flour base, which contains the gluten that imparts pleasant textural properties to the baked products. Wheat and other gluten-containing breads such as rye cannot be tolerated by people with celiac sprue, which is caused by a toxic reaction to gluten proteins (Farrell and Kelly 2002). Rice proteins are devoid of incriminating prolamin proteins such as gliadin (wheat), secalin (rye), and hordein (barley) and therefore rice is an ideal grain to develop foods for celiacs. Rice breads with all rice ingredients have been developed with acceptable texture and flavor and only minor shelf-life issues (Kadan and others 2001; Watanabe and others 2004).

Whole-grain breads are a good source of minerals such as magnesium, iron, and zinc. However, breads made from whole grains also contain phytic acid (*myo-inositol* hexakis [dihydrogen phosphate], which chelates multivalent cationic minerals and renders them insoluble and unavailable for absorption (O'Dell and Savage 1960). Comparison of the 1999 to 2000 Natl. Health and Nutrition Examination Survey (Ervin and others 2004) with the Dietary Reference Intakes reports from the Food and Nutrition Board (Inst. of Medicine 1997, 2000) reveals that most Americans consume less than the Recommended Dietary Allowance (RDA) for magnesium, and most American women obtain less than the RDA for iron. Celiac disease presents a number of clinical conditions such as chronic diarrhea, osteoporosis, and iron deficiency anemia. These deficiencies may be compounded by foods having high amounts of phytic acid such as rice breads containing rice bran (Farrell and Kelly 2002; Kahlon and Smith 2004).

During breadmaking, phytases from the grains and the yeast can degrade the phytate, releasing inorganic phosphate and a series of inositol phosphate intermediates containing fewer phosphate groups (Türk and others 1996). Increasing the amount of yeast from approximately 0.9% to 1.8% of the dry weight of the bread mix increased the amount of phytate hydrolysis in rye bread, resulting in a decrease in the phytate-to-zinc molar ratio from 18 to 10 (Harland and Harland 1980). Zinc bioavailability is predicted to be poor when the ratio is above 15, medium from 5 to 15, and high below 5 (World Health Organization 1996).

Addition of defatted rice bran to the mix has been used to improve the flavor and texture of the rice bread (Kadan and others 2001). Similar to wheat bran, rice bran contains a high amount of phytate (Phillippy 2003). The purpose of the following work was to determine the phytate and minerals in rice breads made with different combinations of yeast and bran in order to evaluate their nutritional quality as a replacement for breads made from grains containing gluten.

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Materials and Methods

Rice bread

Rice bread was prepared as described previously (Kadan and others 2001). The recipes for all breads contained 233-g white flour milled from Cypress long grain rice (Riceland Foods Inc., Stuttgart, Ark., U.S.A.), 6-g salt, 30-g sugar, 4.7-g methocel K4M (Dow Chemical Co., Midland, Mich., U.S.A.), 14-g rice bran oil (Oilseed Intl. Ltd., San Francisco, Calif., U.S.A.), and 256-g water. Nine loaves were prepared in duplicate using 3 levels each of bread machine yeast (Fleischmann's, Fenton, Mo., U.S.A.) and defatted rice bran (Riceland Foods Inc., Stuttgart, Ark., U.S.A.). The yeast levels were 5.11, 10.22, and 15.33 g; the bran levels were 11.71, 23.42, and 35.13 g. Three loaves were prepared on each of 6 d using a factorial arrangement with a completely randomized design. The dry ingredients were mixed in a blender. The oil and water were put into the baking pan of the bread machine (Welbilt Model ABM 3500; Appliance Co. of America, Great Neck, N.Y., U.S.A.). The dry ingredients were added, and the "sweet" program, taking 2 h and 50 min, was started. Baked breads were stored at −20 °C until thawed for analysis.

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Phytate analysis

The crust was removed and the interiors of the loaves were crumbled and dried for 16 h at 85 °C in a gravity convection oven. After grinding with a mortar and pestle, 1-g bread was extracted in duplicate by stirring with 10-mL 0.75 N HCl for 60 min. The extracts were centrifuged 30 min at 50000 × g and 20 °C, and the supernatant solutions were filtered through 0.45 μM Millex®-HV filter units (Millipore Corp., Bedford, Mass., U.S.A.). Ten-microliter aliquots of the filtered solutions were analyzed for phytate by isocratic ion chromatography as described previously (Phillippy and Wyatt 2001).

Mineral analyses

Twenty-microliter aliquots of the sample solutions prepared for phytate determination were analyzed for inorganic phosphate by addition of 180-μL H_2O and 800-μL freshly prepared acetone/5N H_2SO_4/10 mM ammonium molybdate (2:1:1), mixing and measuring the absorbance at 355 nm (Heinonen and Lahti 1981). Breads with the crust removed were sent to Eurofins (Des Moines, Iowa, U.S.A.) for determination of magnesium, iron, and zinc by inductively coupled plasma spectrometry (Horwitz 2000).

Statistical analysis

The duplicate data for phytate and inorganic phosphate from each of the duplicate loaves were combined to calculate a mean and standard deviation ($n = 4$). Magnesium, iron, and zinc data from the duplicate breads were averaged to calculate a mean ($n = 2$). Two-way analysis of variance (ANOVA) was used to determine significant differences at $P < 0.05$ by post hoc comparisons using the Bonferroni correction with the free online calculator at www.graphpad.com.

Results and Discussion

In order to calculate the original amounts of phytate and inorganic phosphates in the bread mixes, the ingredients were individually determined. The phytate and inorganic phosphate contents of the 9 recipes are summarized in Table 1.The rice flour contained 2.54 ± 0.58 μmol/g phytate and 3.6 ± 0.2 μmol/g phosphate. The rice bran had 66.2 ± 3.1 μmol/g phytate and 56.4 ± 3.0 phosphate. The yeast provided zero phytate and 33.4 ± 0.8 μmol/g phosphate.

As expected, the breads containing the highest amount of bran contained more phytate than those with less bran (Table 2). The level of yeast did not have any noticeable effect on the amount of phytate remaining in the breads. However, the levels of inorganic phosphate hydrolyzed by the same level of yeast progressively decreased with the increasing levels of bran (Table 3). The breads with the most bran contained the most inorganic phosphate, but there was also a significant increase in phosphate with increasing levels of yeast (Table 3) at all 3 levels of yeast. The most likely explanation for the different effects of yeast on the phytate and inorganic phosphate is that organic phosphates other than phytate were hydrolyzed. Some of the inorganic phosphate may also have come from phytate, because more than 1 phosphate can be liberated from each molecule of phytate, thus making phosphate a more sensitive indicator of phytate hydrolysis than phytate itself. In whole wheat breads made with fermentation times of 4 and 8 h, Harland and Harland (1980) found that yeast levels of 0.8% of the ingredients resulted in 25% and 34% decreases of phytate, respectively, whereas 1.6% yeast gave phytate losses of only 22% and 28%, respectively, compared to a 0-h control.

Subtracting the amounts of phytate and inorganic phosphate in the breads from the amounts in the original mixes yielded the changes in these compounds. As shown in Table 4, increasing amounts of bran significantly decreased the amount of phytate degradation from an average of 42% to 10%, and the yeast level had no effect. The net decreases in phytate averaged 2.03, 1.49, and 0.87 μmol/g in the low-, medium-, and high-bran breads, respectively. The inverse relationship between phytate degradation and bran level may be explained by the presence in the bran of phytase inhibitors such as high levels of minerals and phytate, which have been shown to inhibit many phytases when present at millimolar concentrations (Konietzny and Greiner 2002). In contrast to phytate, the amount of inorganic phosphate formed did increase with higher yeast concentrations, and the differences between the high and the other levels of yeast were significant at $P < 0.05$ (Table 5). It is obvious that much of the phosphate did not come from phytate because the phosphate increases at the higher bran levels were much greater than 6 times the molar decreases in phytate ($InsP_6$). In general, the inorganic phosphate roughly doubled with increases ranging from 86% to 123%.

Table 1 — Contents of phytate and inorganic phosphate in rice bread recipes

Bread bran/yeast	Flour phytate µmol	Bran phytate µmol	Total dry weight g	Total phytate µmol	Phytate µmol/g
1/1	699	795	305	1494	4.90
1/2	699	795	310	1494	4.82
1/3	699	795	315	1494	4.74
2/1	699	1590	316	2289	7.24
2/2	699	1590	321	2289	7.13
2/3	699	1590	326	2289	7.02
3/1	699	2385	328	3084	9.40
3/2	699	2385	333	3084	9.26
3/3	699	2385	338	3084	9.12
Bread bran/yeast	**Flour phosphate µmol**	**Bran phosphate µmol**	**Yeast phosphate µmol**	**Total phosphate µmol**	**Phosphate µmol/g**
1/1	850	680	174	1704	5.59
1/2	850	680	347	1879	6.05
1/3	850	680	521	2051	6.51
2/1	850	1361	174	2385	7.55
2/2	850	1361	347	2558	7.97
2/3	850	1361	521	2732	8.38
3/1	850	2041	174	3065	9.34
3/2	850	2041	347	3238	9.72
3/3	850	2041	521	3412	10.09

Table 2 — Phytate content of rice breads[a]

	Bran level		
	1	2	3
Yeast level			
1	2.75 ± 0.18[b]	5.72 ± 0.12[c]	8.67 ± 0.09[e]
2	2.94 ± 0.11[b]	5.80 ± 0.07[c]	8.15 ± 0.17[d]
3	2.67 ± 0.19[b]	5.40 ± 0.16[c]	8.34 ± 0.39[de]

[a]Data are means ± standard deviation and expressed in µmol per gram dry weight.
[b–e]Means without the same superscript are significantly different at $P < 0.05$.

Table 3 — Inorganic phosphate content of rice breads[a]

	Bran level		
	1	2	3
Yeast level			
1	11.8 ± 0.7[b]	14.4 ± 0.1[c]	17.4 ± 0.3[d]
2	12.6 ± 0.6[b]	15.7 ± 1.1[c]	18.5 ± 0.3[d]
3	14.5 ± 0.3[c]	18.3 ± 0.3[d]	20.7 ± 1.3[e]

[a]Data are means ± standard deviation and expressed in µmol per gram dry weight.
[b–e]Means without the same superscript are significantly different at $P < 0.05$.

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There have been several reports of phytate degradation in breads. The rate of phytate hydrolysis is determined in part by the amount of phytase contributed by the grain (Türk and others 1996), the yeast (Harland and Harland 1980; Türk and others 1996), or phytase additives (Türk and Sandberg 1992; Porres and others 2001; Haros and others 2001). Other critical factors in the loss of phytate include the time the dough is allowed to ferment before enzymes and microorganisms are activated by the heat of baking (Harland and Harland 1980; Türk and Sandberg 1992) and the concentration of phytase inhibitors and the pH of the dough (Larsson and Sandberg 1991). Recently, Watanabe and others (2004) observed 45% to 54% phytate degradation in breads where 30% of the wheat flour was substituted with brown rice flour. Both rice flour and stabilized rice brans were expected to have no significant phytase activities.

The phytate concentrations in the finished rice breads ranged from 2.67 to 8.67 μmol/g (Table 2). Plaami and Kumpulainen (1995) found 2.62 μmol/g $InsP_6$ in wheat and rye bread and 8.26 μmol/g $InsP_6$ in wheat and oat bread. In their breads, $InsP_6$ was the predominant inositol phosphate, although substantial amounts of $InsP_5$, $InsP_4$, and $InsP_3$ were also observed in the former. Phillippy and others (1988) also observed mostly $InsP_6$ in white wheat bread, but Ferguson and others (1993) quantified more $InsP_5$ than $InsP_6$ in a similar product. In the present study, only $InsP_6$ was measured, but it is very likely that appreciable amounts of $InsP_5$ were also present after fermentation. This is important, and will be discussed below, because $InsP_5$ affects the bioavailability of minerals similarly to $InsP_6$ (Fox and Tao 1989; Sandberg and others 1999).

In a preliminary experiment, it was determined that 100-g wet weight (equivalent to about 4 slices) of the rice breads contained 3% or more of the RDAs for iron, magnesium, and zinc, but only approximately 1% of the RDA for calcium (results not shown). Therefore, the amounts of the former 3 minerals were determined in the breads. Breads with the highest amounts of bran had more than double the amounts of magnesium compared to those with the lowest (Table 6). The iron content was approximately double in the high-bran compared to the low-bran breads, and zinc showed smaller percent increases with the bran. Small contributions to the iron and zinc but not the magnesium contents of the breads were made by the yeast. The phytate-to-zinc molar ratios were between 5 and 10 for the low-bran breads, which would suggest a medium level of bioavailability (World Health Organization 1996). However, since $InsP_5$, which affects mineral availability similar to $InsP_6$, was not measured, some of their zinc bioavailabilities could be lower, as would be expected for the medium- and high-bran breads with ratios above 15. Nevertheless, the higher amounts of zinc in the high-bran breads may compensate for their lower bioavailabilities, because the amount of bioavailable zinc was found to be inversely related to the degree of milling of rice fed to rats (Hunt and others 2002). Similarly, Levrat-Verny and others (1999) observed an increase in the total absorption of zinc and iron in the presence of wheat bran in rats, and Sandström and others (2000) found that oat bran increased the total absorption of zinc in humans. Therefore, rice breads made with bran may be a good nutritional source of minerals such as magnesium, iron, and zinc despite their phytate content, because they contain high levels of these minerals. Insufficient studies on phytate-to-iron or phytate-to-magnesium ratios have been conducted to allow meaningful predictions for the availability of those minerals.

Finally, the value of the residual phytate in the breads should not be overlooked. Phytate has numerous positive nutritional attributes that belie its reputation as an antinutrient (Phillippy 2003). Because the targeted consumers of rice bread are individuals with malabsorption due to gluten sensitivities, the numerous associated conditions and complications (Farrell and Kelly 2002) may be ameliorated by the power of phytate to act as a possible antioxidant or anticarcinogen. Rice bread may be an alternative for those who are sensitive to and cannot adequately absorb nutrients from wheat bread. Phytic acid is a chemical constituent of rice, occurring predominantly in the bran that binds to minerals and decreases their bioavailability. The amounts of rice and yeast in rice bread were varied to determine the best rice bread recipes from nutritional standpoint. Rice bread appears to be good source of minerals such as magnesium, iron, and zinc that could improve the nutrition of the 2 to 3 million people in the United States who are intolerant to wheat bread.

Table 4 — Phytate decreases in rice breads compared to the ingredients[a]

	Bran level		
	1	2	3
Yeast level			
1	2.15 ± 0.18[b]	1.52 ± 0.12[cd]	0.73 ± 0.09[f]
2	1.88 ± 0.11[bc]	1.32 ± 0.07[de]	1.11 ± 0.17[ef]
3	2.07 ± 0.19[b]	1.62 ± 0.16[cd]	0.78 ± 0.39[f]

[a]Data are means ± standard deviation and expressed in μmol per gram dry weight.
[b–f]Means without the same superscript are significantly different at $P < 0.05$.

Table 5 — Inorganic phosphate increases in rice breads compared to the ingredients[a]

	Bran level		
	1	2	3
Yeast level			
1	6.26 ± 0.68[b]	6.90 ± 0.10[bcd]	8.04 ± 0.33[de]
2	6.55 ± 0.61[bc]	7.78 ± 1.09[cde]	8.78 ± 0.29[ef]
3	7.99 ± 0.32[de]	9.89 ± 0.29[fg]	10.59 ± 1.25[g]

[a]Data are means ± standard deviation and expressed in μmol per gram dry weight.
[b–g]Means without the same superscript are significantly different at $P < 0.05$.

Conclusions

The amount of phytic acid in rice breads is directly related to the level of rice bran, and the amount of phytic acid degraded during breadmaking decreases with its increased concentration at all 3 yeast levels. Yeast levels from 1.6% to 4.7% of the dry ingredients do not significantly affect the residual amount of phytate. The phytate-to-zinc molar ratios were between 5 and 10 for low-bran breads, suggesting good bioavailability of minerals in rice breads. Rice bread is a tasty and nutritious food that is a good dietary source of minerals, including magnesium, iron, and zinc, for people who cannot tolerate wheat bread.

Table 6 — Mineral contents of rice breads

Bread Bran/yeast	Iron μmol/g	Magnesium μmol/g	Zinc μmol/g	Phytate:Zinc mol:mol
1/1	0.18[a]	25.8	0.29	9.5
1/2	0.20	25.3	0.33	8.9
1/3	0.22	26.8	0.40	6.7
2/1	0.26	39.9	0.37	15.5
2/2	0.28	41.4	0.37	15.7
2/3	0.31	41.6	0.43	12.6
3/1	0.38	63.9	0.38	22.8
3/2	0.38	61.3	0.43	19.0
3/3	0.41	65.7	0.47	17.7

[a]Data are averages of 2 values and expressed on a dry-weight basis.

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